III. REMARKS

Claim Status

Claims 14-27 are in the case, claims 14-21 and 27 stand rejected; claims 22-26 are withdrawn.

Claim Objections

Claim 27 stands objected to under 37 CFR 1.75 as being a substantial duplicate of claim 14. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim.

Applicant traverses this objection as being premature as neither of the claims has been allowed.

Claim Rejections - 35 USC § 112, first paragraph, written description

The new matter rejection of claim 16 under 35 USC 112, first paragraph, as failing to comply with the written description requirement based on the limitation "the suspension comprises cells which present the protein fragment bound to MHC class I or class II molecules." is maintained for reasons of record.

The examiner states that, contrary to Applicant's assertion, support for said limitation does not appear in the specification at page 7, second paragraph nor does this phrase appear in the specification or original claims as filed. The cited portion of the specification recites "said suspensions containing T cells contain cells which present the protein

fragment essentially with MHC I or II molecules...". the examiner argues that this phrase does not provide support, either explicit or implicit, for the limitation recited in claim 16. Therefore this limitation is new matter.

Applicant respectfully traverses this ground for rejection.

Claim 16 was previously amended by eliminating the word "essentially in a state" from the phrase "…cells which present the protein fragment essentially in a state bound to MHC class I or class II molecules.

The first issue is the meaning that attaches to the deleted phrase. The second issue is the scientific background relating to the meaning of the deleted phrase.

As to the plain English meaning of "essentially in a state", Webster's New World Dictionary, College Edition defines "essential" as something "basic; inherent". The same dictionary defines "essentially" as "in a characteristic manner". So the original plain English language meaning of the phrase "essentially in a state" means that the fragment is "basically in a state" or "inherently in a state" or "characteristically in a state" bound to the MHC molecules. The removal of the phrase in response to the examiners rejection was an attempt to use a different form of words to describe the same thing. Applicant did not intend to and strongly would argue that it did not change an *iota* of the meaning of the full clause but simply changed the form of words to describe the same thing.

The second issue relating to the clause and the deletion of the phrase is the scientific background relating to the meaning of the deleted phrase.

The phrase “Cells which present the protein fragment essentially in a state bound to class-I or class-II molecule" is a description of the cells that must be contained in the suspension. It is not an instruction for which further guidance needs to be given. Cells that qualify for this description are professional APC (e.g. monocytes and B- cells) for class II MHC binding peptides and all blood cells expressing MHC-class-I for class-I binding peptides (e.g. monocytes, lymphocytes, granulocytes to some extent etc.).

All of these cells possess classic MHC molecules and will present peptides essentially in a state bound to them (which of the blood cells possess MHC molecules was known a long time ago, (see Ivan Roitt, Immunology, Churchill Livingstone/Gower, 1985, page 4.1).

Anyone skilled in the field knows that a PBMC preparation, for example, will contain a large proportion of the required cells. And anyone skilled in the field knows that cell lines not expressing classic MHC molecules would not be suitable for this purpose.

“Essentially” indicates that this state of being bound to MHC is the very nature of peptides that are presented.

Several non-classic MHC/molecules are known to present small molecules that can be recognized by a variety of receptors on other cells (for example, HLA-E can present peptides from viruses to a T-cell subset: C. Romagnani et al., Human Immunology, Volume 65, Issue 5, May 2004, pages 437-445.)

The existence of such non classical ways of presentation was known long before applicants filed this application (see review in Brand et al., Current Opinion in Immunology, 1999, 11:100-108)

In particular reference 4 quoted in the Brand et al. review article, [Braud V. et al., The human major histocampatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9, Eur. J. Immunol. 1997, 1164-1169] and reference 5 quoted in the Brand et al. review article, [Lee, N. et al, HLA-E surface expression deprends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences, J Immunol, 1998, 160:4951-4960] and others as quoted in the same review and published before 1997, are relevant. References 4 and 5 show that certain signal sequence derived peptides are normally found to be presented on HLA-E molecules, suggesting that any peptide with the right anchor residues may be loaded onto such molecules.

The mere occurrence of this kind of non-classic presentation cannot be excluded for the disclosed peptides but will only be a very minor phenomenon in practice, concern a very small number of non-classic MHC molecules on presenting cells, concern a very small target cell subset (much smaller than the CD8+ or CD4+ classic- MHC:restricted T-cell subsets, which together typically represent >95% of all T-cells) and is not the mechanism of interest for the present application.

Thus, from a scientific basis the language “essentially in a state bound to” properly identifies the meaning intended and the proper scope of the present invention. So also does the

language “bound to” as the two have the same meaning within the terms of this specification and claims.

Applicants have retained the simplified language “bound to” in claim 16 but, at the request of the examiner, are willing to revert to the original language as the two have identical meanings.

***Claim Rejection – 35 USC§ 112,* second paragraph, indefinite**

Claims 14-21 and 27 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The examiner states that claim 14 is rendered vague and indefinite by the use of the phrase "cleaving the amino acid sequence of said antigen" in step c). It is unclear how a sequence can be cleaved as they constitute an abstraction.

Claim 14 has been amended to clarify that it is the antigen that is being cleaved thus obviating this ground for rejection.

The examiner states that claim 14 is rendered vague and indefinite by the use of the phrase "at least one activation marker expressed or expression enhanced due to the T cell stimulation by the protein fragment or fragments which has been induced or expression enhanced by the protein fragment or fragments..." and that is unclear how said fragments can be "induced or expression enhanced" by itself. Moreover, it is unclear what is meant by the term "expression-enhanced". Consequently, it is impossible to determine the metes and bounds

of the claimed invention.

Claim 14 step e) (ii) has been amended to clarify that an increased amount of marker is present and is caused by T cell stimulation thus obviating this ground for rejection.

The examiner states that claim 14 and 27 are rendered vague and indefinite by the use of the phrase "...taken up by the major to his compatibility antigen (MHC) molecules...".

This typo has been corrected thus obviating this ground for rejection.

The examiner states that claim 14 is rendered vague and indefinite by the use of the phrase "selection and proliferation". It is unclear to the examiner what cell population said phrase is drawn to.

The examiner states that there is insufficient antecedent basis for the limitation "the suspension"" in claim 16.

Applicants believe there is sufficient basis as this refers to the suspension specified in claim 14 step d); however applicant has further clarified the point by amending claim 16 to recite the step of claim 14 where the antecedent language appears thus obviating this ground for rejection.

Applicant respectfully traverses this ground for rejection.

With respect to Claim 14 the examiner maintains that the incubation time of peptides ("protein fragments") with T-cell-containing cell suspensions has not been sufficiently defined to allow reproduction of the experiment by anyone skilled in the art without undue experimentation.

Applicants respectfully disagree.

In Claim 1 of the application, this incubation time has been described as being "sufficiently long" to allow uptake of peptides into the binding groove of MHC molecules on one hand, and "sufficiently short" to avoid proliferation of T-cells stimulated by the presence of said peptides in the MHC binding groove.

Applicants have supplied citations confirming that anyone skilled in the set-up of cell culture experiments is aware that proliferation typically occurs not before 24 hours of stimulating T-cells.

Applicants made a mistake in the wording of their response that is unfortunate. However, does not change the facts of what was known at the time of filing nor what is written in the application. The mistake in applicant's response resulted from word-processing (copy/paste action) as a consequence of which the phrase "this time can be six hours" could be understood to refer to the time required for proliferation, whereas the application specification says that the incubation time can be six hours (page 13, 3rd line from bottom), a time that would be "sufficiently long" to allow uptake of peptides into the binding groove of MI IC molecules on one hand, and "sufficiently short" to avoid proliferation of T-cells stimulated by the presence of said peptides in the MHC binding groove). ["Selection and proliferation accompanied by specific elimination of particular T cells do not occur in the method according to the invention due to the short incubation times." Specification, page 5, second paragraph]

The examiner correctly noted this apparent contradiction in applicants' reply which he interpreted as suggesting applicants "give support of the lack of direction with regard to the time between antigen uptake and onset of proliferation."

Applicants would like to repeat that, as a statement of scientific fact, no proliferation can possibly occur within six hours and that this was current knowledge at the time of filing (and long before). Neither Applicants nor the examiner have provided any citations that teach otherwise. All provided citations support the well known fact that proliferation does not occur earlier than 24 hours after antigen-uptake, which is, indeed, one of the basic facts of human immunology.

Proliferation assays were among the earliest immunological assays performed and their basic kinetics had been established decades ago (publications from 1960's about this topic are available in PubMed).

As a consequence, examiner has rejected not the actual claim or its specification but the result of an unfortunate word-processing error in applicants' response letter.

Conceding this misunderstanding, the literature quoted, and the fact that six hours is specified as a possible incubation time, applicants respectfully suggest that the error occurred solely in the response, that no conflicting statements were made in the application and that the specification and claims provide sufficient guidance and that the examiner should look to the four corners of the applicant to appreciate the clear meaning.

In response to the examiner's prior rejection, and solely to clarify the meaning and intention and not to make any substantive change or impose any limitation on the original meaning of the claims, applicants amended original claim 14 by eliminating "specific" and "particular" from the last sentence of section f) of this claim.

> ....; and the incubation time of the suspension containing T-cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T-cells do not occur.

This original wording reflected two basic facts.

1. Specific elimination

Specific elimination of the T cells can occur through several mechanisms. Following successful activation of T-cells, a proportion of activated T-cells will naturally proliferate. Proliferation involves the synthesis of DNA, which will result in an increased uptake of nutrients, growth factors, and cytokines such as IL-2, required for cell survival.

In a limited culture space with a limited supply of said elements, T -cells that are more efficiently activated (T-cell clones with high avidity of the TCR for the NHC-peptide-complex) will have a proliferation advantage and exhibit higher consumption of nutrients, growth factors and cytokines. They will thus deprive other cells of these critical components.

By contrast, less efficiently activated T-cells (for example lower avidity clones) have a disadvantage and may not be able to take up sufficient amounts of nutrients, growth factors, and cytokines. The affinity/avidity model of T-cell activation was introduced in 1997 (Ward, ES, and Qadri, A; Current opinion in Immunology, 1997, 9:97-1061). In this sense, specific elimination occurs following specific activation, and the particular cells that are lost are those of lower affinity.

Because activation is specific (i.e. limited to those T-cells recognizing antigens like peptides presented in an MHC context) elimination of T-cells as a result of starvation following successful activation is equally specific. For example, if flu-specific peptides are used for stimulation, flu-specific T-cells may die in this way, but not tetanus specific T-cells. Using an incubation time of less than 24 hours, such effects are unlikely to occur. The shorter the incubation time, the less likely they are to occur.

2. Particular T-cells

"Particular" refers to those T-cells which, because of their lower avidity to the MHC-peptide complex will not be as strongly or effectively activated as T-cells of higher avidity to the WIC-peptide-complex.

The original wording was a reflection of these differences. In any case, "particular" is equivalent to "specific", meaning that these cells can be defined and are different by certain criteria than other cells in the culture. Even the fact of dying as such makes them particular, which, from applicants' point of

view turns this discussion into a tautology. As soon as the cells die they are particular for that very reason, since the others do not. Applicants respectfully suggest that this is a semantic game, not a serious reason for rejecting a claim.

Applicants have observed that T-cells can require massive amounts of the survival factor IL-2. Unless IL-2 is added in sufficient amounts, T-cell responses (synthesis of cytokines) can be observed after 6 hours but not after 48 hours (proliferation) because activated cells are starved. That IL-2 needs to be added to T-cell cultures to prevent loss off-cells during proliferation was already known in the early 90s. The addition of IL-2 for the culture of CMV specific T-cells stimulated by peptides was established long before the filing date of our application (already in 1991). Mark Wills in 1996 [Wills et al, 1996, J Virol, 70:7569-79, PDF supplied] used a limiting dilution proliferation assay to derive different clones from the stimulation by individual peptides. This illustrates further what applicants meant when they referred to specific elimination of particular cells.

In sum, applicants believe the language of the original claim and the language of the amended claim define the same scope and are equivalent. Applicants' have not reintroduced the original language into claim 14 but upon request of the examiner, would be willing to do so, based on their understanding that the two forms are identical in meaning.

Claim Rejections - 35 USC § 102

Claims 14-19, 21 and 27 stand rejected under 35 U.S.C. 102(a) as being anticipated by Yanagisawa et al. (International Immunology, 1997, Vol. 9 No. 2, pages 227-237).

The instant claims are drawn to methods for identification of T-cell stimulating protein fragments. At page 4 of applicants' specification, under the subheading "Advantages" applicant states:

> "The main advantage of this method according to the invention is that a protein segment with a known sequence can be identified as a T-cell stimulating protein fragment with a very short period of time and, as compared with the conventional method, with very little expenditure. The time between the first incubation of T cells and the flow cytometric evaluation can be six hours." [emphasis supplied]

Yanagisawa et al. disclose a method that requires a 72 hour culture period [page 228, col. 2 next to last line], a 24-48 hour culture period [page 229, col. 1, lines 5-6] and a 4 day culture period [page 229, col. 1, lines 9-11].

As stated by applicant's specification, at page 5, lines 11-13:

> "Selection and proliferation accompanied by the specific elimination of particular T cells do not occur in the method according to the invention due to the short incubation times."

The examiner states that with regard to the limitation that the incubation time be sufficiently short so that

selection, proliferation and the specific elimination of stimulated T cells does not occur, it is deemed, in absence to evidence to the contrary, that since the active expression of cell surface markers are measured on the stimulated T cells, said cells could not have been specifically eliminated.

Applicant respectfully disagrees.

Applicants believe that the discussion, infra, regarding the meaning of “specific” and “particular” and the references there cited provide irrefutable evidence for the correctness of the statements in the specification and claims.

In conclusion since the time period specified by applicant in his method is 4 times shorter than the shortest time period specified by Yanagisawa et al., and because of the obvious advantage of performing the entire procedure within one working day, applicant have demonstrated a significant and useful difference between his invention and the prior art process exemplified by Yanagisawa et al.

Claim Rejections - 35 USC § 103

Claims 14-21 and 27 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Yanagisawa et al. (International Immunology, 1997, Vol. 9 No. 2, pages 227-237) and Picker et al. (Blood, 1995, Vol. 86 No. 4, pages 1408-1419).

The disclosure of Yanagisawa et al. is discussed above.

As the examiner acknowledges, Yanagisawa et al. differs from the instant invention in that the cytokine levels are measured by ELISA.

Picker et al. disclose a multiparameter flow cytometric

assay. The examiner therefore argues that it would have been obvious for one of ordinary skill in the art at the time of the invention to use the flow cytometry method of Picker et al. in the epitope mapping method of Yanagisawa et al. in order to take advantage rapid ability to determine the functional potential (i.e. response) of phenotypically distinct T cell subsets and that one would have had a reasonable expectation of success since Picker et al. disclose that the "simplicity and rapidity" of their detection technique coupled with the widespread availability of flow cytometers and T cell phenotyping antibodies suggest that their technique could be broadly applicable to the evaluation of immune status.

Applicant respectfully disagrees.

Essentially the examiner is suggesting that it would be obvious to one skilled in the art to use flow cytometric techniques rather than the ELISA technique of Yanagisawa et al. when that suggestion flies in the face of Yanagisawa et al.'s express disclosure.

Yanagisawa et al. was clearly aware of flow cytometric techniques and utilized such techniques to analyze the expression of cell surface antigens [page 229, first col., subheading "Flow Cytometry"]. If it were obvious, as suggested by the examiner, to utilize flow cytometric techniques as utilized by applicant, then Yanagisawa et al. would certainly have utilized such techniques as 1] Yanagisawa et al. was aware of the usefulness of flow cytometry, and 2] flow cytometry provides, in conjunction with the other aspects of applicants process, significant benefits.

That Yanagisawa et al. did not use such a technique when it was available to him demonstrates that it is only with

impermissible hindsight that one would combine the two teachings to reach applicants invention.

Favorable reconsideration is requested.

Conclusion

Applicant believes these remarks and the claim amendments are sufficient to obviate the grounds for rejection presented in the outstanding office action and respectfully requests allowance of the pending claims. Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,

NORRIS McLAUGHLIN & MARCUS, P.A.

By: /Serle Ian Mosoff/
Serle Ian Mosoff
Attorney for Applicant(s)
Reg. No. 25,900
875 Third Avenue - 18th Floor
New York, New York 10022
Phone: (212) 808-0700
Fax: (212) 808-0844